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镉诱导萝卜幼苗活性氧产生、脂质过氧化和抗氧化酶活性的变化

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摘　要：通过水培试验，研究 Cd^{2+} 胁迫对萝卜幼苗活性氧的产生、脂质过氧化和抗氧化酶活性的影响。超氧阴离子($O_2^{\overline{\cdot}}$)的产生速率和丙二醛(MDA)的含量与对照相比有不同程度的增加，表明 Cd^{2+} 胁迫能导致萝卜体内的氧化胁迫；超氧化物歧化酶(SOD)的活性，随着 Cd^{2+} 浓度提高，首先明显上升，然后逐渐下降，甚至低于对照，叶片过氧化氢酶(CAT)的活性明显增加，根系 CAT 活性则减少，根系以及较高浓度 Cd^{2+} 处理后期叶片谷胱甘肽还原酶(GR)的活性均显著增加。推测：胁迫初期可能主要由 SOD 和 CAT 发挥抗氧化作用；后期由于抗坏血酸—谷胱甘肽(AsA-GsH)循环途径的激活，以及还原型谷胱甘肽(GSH)和植物络合素(Phytochelatins，PCs)的合成，可能在清除活性氧或者直接螯合 Cd^{2+} 中起作用。

关键词：镉；氧化损伤；抗氧化酶；水培；萝卜

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Cadmium-induced superoxide anion generation, lipid peroxidation and changes of antioxidant enzyme activities in radish seedlings

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Abstract: While seedlings of radish raised in increasing contents of Cd^{2+} in hydroponic system, increment in ratio of superoxide dismutase(SOD)/catalase(CAT) and levels of superoxide anion($O_2^{\overline{\cdot}}$) and lipid peroxides were observed; 125 μmol/L Cd^{2+} treatment resulted in a gradual elevation in SOD activity; while at Cd^{2+} level of 250 and 500 μmol/L, SOD activity considerably increased at first, then declined to even lower than that of the control. CAT activity showed enhancement in leaves whereas decrease in roots. Cd^{2+} induced an obvious elevation in GR activity in both roots and leaves. A marked elevation in GR activity suggests that ascorbate-glutathione(AsA-GsH) cycle may be activated to scavenge AOS and the synthesis of reduced glutathione (GSH) may be stimulated for subsequent synthesis of phytochelatins(PCs) to chelate Cd^{2+} directly.

Key words: cadmium; oxidative stress; antioxidant enzymes; hydroponic culture; radish

Even under natural conditions of growth and development, plants face constant risk from active oxygen species(AOS), including $O_2^{\overline{\cdot}}$, hydrogen peroxide(H_2O_2), hydroxyl radical($\cdot OH$) inevitably generated via number of metabolic pathways. AOS play important roles in plant's defence system against pathogens, mark certain developmental stages such as lignification and other cross-linking

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processes in the cell wall and act as intermediate signaling molecules to regulate the expression of genes, while excess AOS can damage membrane lipids, proteins, pigments and nucleic acids, resulting in dramatic reduction of productivity, finally the death of plants (Hegedüs *et al.*, 2001). To avoid oxidative damage, plants have evolved various protective mechanisms, one of which is the enzymatic antioxidant system operating with the simultaneous and sequential action of number of enzymes such as SOD, CAT, peroxidases (POD) and GR.

Heavy metals can cause molecular oxidative damage to plants either directly or indirectly through the formation of AOS (Gallego *et al.*, 1996; Cho *et al.*, 2000; Malecka *et al.*, 2001; Shah *et al.*, 2001).

Understanding the biochemical detoxification strategies that plants adopt against oxidative stress is a key to manipulate heavy metal tolerance in plants. Cadmium (Cd^{2+}) is phytotoxic strongly and can cause growth inhibition and even plant death. Some studies related to change of antioxidant enzyme activities and AOS level under Cd^{2+} stress have been carried out (Yan *et al.*, 1997; Luo *et al.*, 1998; Wang *et al.*, 2002; Ren *et al.*, 2002; Xu *et al.*, 2001), however, there are few researches analyzing entirely the change of AOS, MDA content and activities of antioxidant enzyme in roots and leaves of plants.

Radish is a heavy metal tolerant plant. The aim of this study was to investigate the responses of $O_2^{\bar{\cdot}}$ generation, lipid peroxidation and SOD, CAT, GR activities to Cd^{2+} treatment in radish seedlings and afford general referenced evidence for phyto-remediation of soil contaminated by heavy metal.

1 Materials and Methods

1.1 Plant culture and treatment conditions

Seeds of three radish (*Raphanus sativus* L.) varieties were surface sterilized with 3.5% NaClO for 20 min and rinsed thoroughly with distilled water, after 3 d germination on moistened filter paper in dark at 25 ℃ with humidity of 70%～80%, the seeds were transferred to a greenhouse maintained at 26 ℃/20 ℃ day/night with 70%～80% humidity and a 16 h photoperiod at 300 $\mu molm^{-2}s^{-1}$ in an aerated hydroponic system in pots containing 1.5 L Hoagland nutrient solution replaced twice a week.

A small experiment on each variety was conducted with ten-day old plants in nutrient solution containing 250 μmol/L Cd^{2+} for 2 d. Based on the growth of these varieties, "No13 Jinhan brand" was selected as material. After growing in the nutrient solution for 20 d, plants were subjected to 0, 125, 250, 500 μmol/L Cd^{2+} treatment, which were labled as Cd0, Cd1, Cd2, Cd3 treatment respectively. Roots and leaves from each treatment were collected at 0, 12, 36, 60, 96 h and stored at -40 ℃ for further analysis.

Experiments were performed in triplicate and the results are the means ± S.D (standard deviation). The analysis of significant difference between control and each treatment were performed using SPSS statistical software. $P \geqslant 0.05$, $P < 0.05$ and $P < 0.01$ indicates respectively that difference is not significant, significant and quite significant.

1.2 Enzyme extraction and assays

The following steps were carried out at 4 ℃. The root or leaf tissue (3∶1 buffer volume∶fresh weight) was homogenized in a pestle and mortar with 100 mmol/L, pH7.5 potassium phosphate buffer containing 1 mmol/L Na_2-EDTA, 3 mmol/L DL-dithiothreitol, 5% (W/V) insoluble polyvinylpyrro-lidone. The homogenate was filtered through muslin cloth and centrifuged at 10 000 × g for 30 min and the supernatant was kept in separate aliquots at -40 ℃, prior to CAT, SOD and GR analysis.

Content of $O_2^{\bar{\cdot}}$ was determined as described by Wang (1990); SOD activity were estimated accor-ding to Cho *et al.* (2000); CAT and GR activities were assayed as described by Vitória *et al.* (2001); MDA content was assayed according to Gallego *et al.* (1996).

2 Results and discussion

2.1 Effect of cadmium on rate of $O_2^{\bar{\cdot}}$ generation

Study has demonstrated that Cd^{2+} can lead to an elevation in $O_2^{\bar{\cdot}}$ generation (Shah *et al.*, 2001).

The change of rate of O_2^- production is presented in Fig. 1. O_2^- generating rate elevated with the increment of Cd^{2+} content especially in roots, with the increase in time of Cd^{2+} treatment it increased at first, then declined, at last elevated again. the maximum was 1.77, 2.14 times higher in leaf at 36 h whereas 2.30, 2.65 times higher in root at 12 h Cd^{2+} treatment respectively than that of control. Statistical analyses indicated that the differences of O_2^- level in both leaves and roots were not significant, significant and quite significant respectively under Cd1, Cd2 and Cd3 treatment compared to Cd0 treatment.

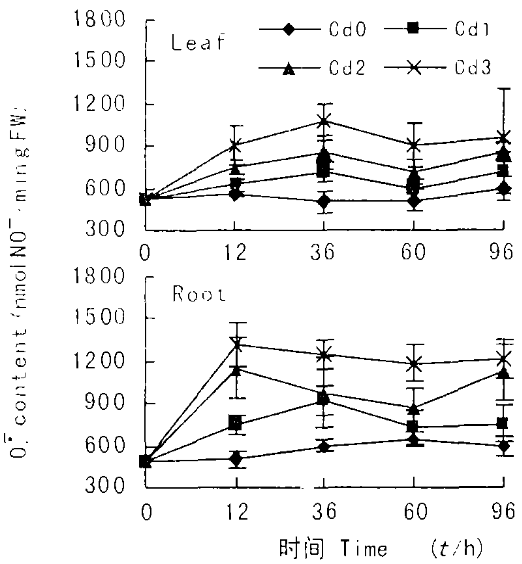

Fig. 1 Effect of Cd^{2+} stress on generation rate of O_2^- of radish seedlings

2.2 Effect of cadmium on SOD activity

As it is shown in Fig. 2, Cd1 treatment resulted in a gradual elevation in SOD activity; while under Cd2, Cd3 treatment SOD activity considerably increased at first, then declined to even lower than that of controls. The differences of SOD activity between Cd1, Cd2, Cd3 and Cd0 treatment did not reach significant levels. Compared to 0 h Cd^{2+} treatment, the differences were quite significant at 36 h in leaves and 12 h Cd^{2+} treatment in roots, while at 36, 60 h Cd^{2+} treatment. it was only significant.

2.3 Effect of cadmium on GR activity

GR can be activated relatively more in roots than in leaves of Cd^{2+}-treated pea plants (Dixit *et al.*, 2001). Fig. 3 indicates that a significantly increase in GR activity was recorded in roots and only under higher Cd^{2+} level treatment could an obvious elevation in GR activity be detected in leaves. 1.11, 2.11, 2.89 times increase in roots whereas 0.50, 1.67, 2.0 times increase in leaves in GR activity was noted after 96 h Cd1, Cd2, Cd3 treatment. Cd3 treatment leaded to a quite significant difference in GR activity in both roots and leaves, while Cd2 treatment caused a significant difference only in roots. Compared to 0 h Cd^{2+} treatment, only after 96 h Cd^{2+} stress had a significant difference in roots GR activity.

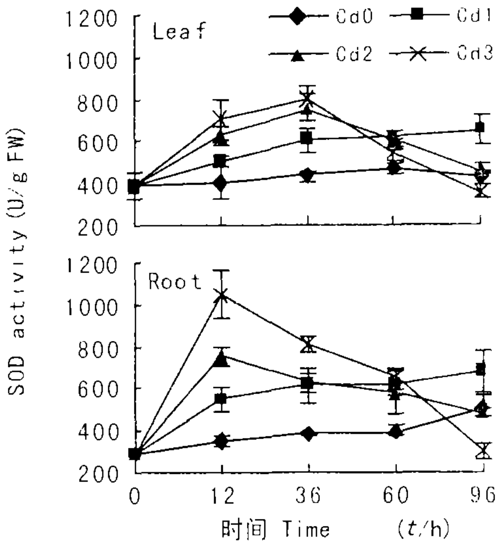

Fig. 2 Effect of Cd^{2+} stress on SOD activity of radish seedlings

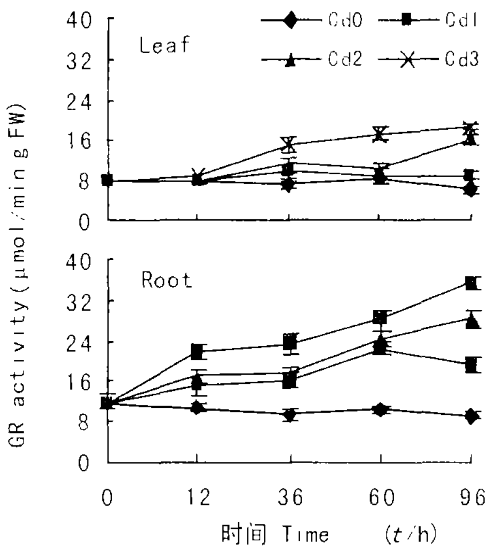

Fig. 3 Effect of Cd^{2+} stress on GR activity of radish seedlings

2.4 Effect of cadmium on CAT activity

CAT, located in peroxisomes, mitochondrial and cytosol, can scavenge H_2O_2 without co-substrates(Hegedüs *et al.*, 2001). The change of CAT activity is showen in Fig. 4. With increase in time of Cd^{2+} stress, an apparent increase followed by a light decrease was reported in CAT activity in leaves; whereas a concomitant decrease in roots CAT activity was observed. Compared to Cd0 treatment, Cd2, Cd3 treatment leaded to a significant difference of CAT activity in leaves, whereas a significant and quite significant difference respectively in roots. Compared to 0 time of Cd^{2+} treatment, the difference which was not significant in roots reached quite significant levels at 36 h and was significant at other time of Cd^{2+} treatment in leaves.

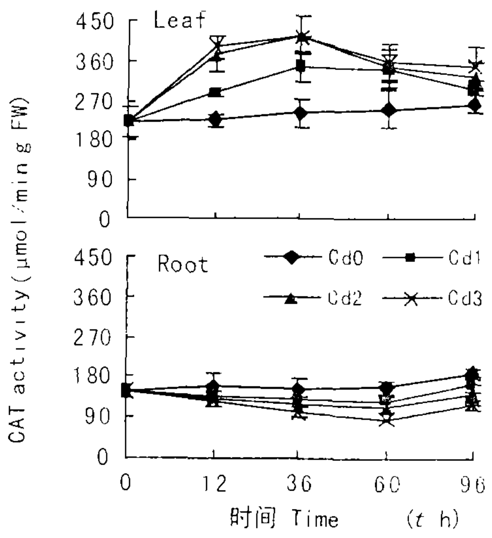

Fig. 4 Effect of Cd^{2+} stress on CAT activity of radish seedlings

2.5 Effect of cadmium on lipid peroxidation

Though antioxidant system can protect farthest plant against oxidative damage, the protection capacity is limited. Under serious stress condition, ·OH can be formed through $O_2^{\cdot-}$ reaction with H_2O_2 and lead to lipid peroxidation. Enhanced lipid peroxidations have been reported under heavy metals stress(Cho *et al.*, 2000; Shah *et al.*, 2001; Luo *et al.*, 1998; Chaoui *et al.*, 1997). The level of lipid peroxides was measured in terms of MDA content(Fig. 5). With increase in Cd^{2+} stress level and time, a gradual increase in MDA level was observed. Compared to Cd0 treatment, only under Cd3 stress could a significant difference of MDA level be seen. The difference reached significant and quite significant levels respectively at 36 h and 60, 96 h Cd^{2+} treatment.

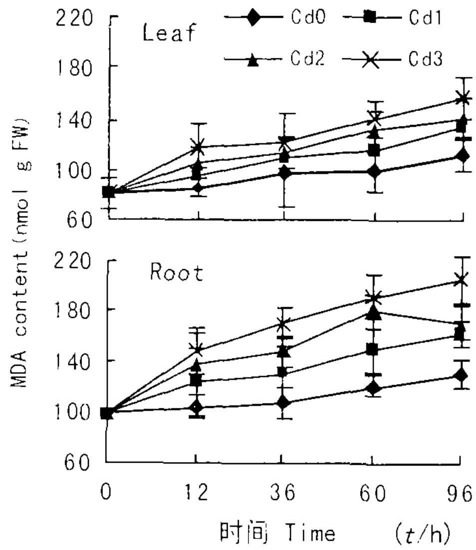

Fig. 5 Effect of Cd^{2+} stress on MDA level of radish seedlings

3 Discussion

Though $O_2^{\cdot-}$ is rapidly dismutated either non-enzymically or via SOD to H_2O_2 and the half life time is less than a second, increase in $O_2^{\cdot-}$ generation under pathogen attack, salinity and signification is also observed and it is associated with either activation of NAD(P)H oxidase or apoplastic peroxidase(Shah *et al.*, 2001). Cd is not a redox metal like Cu and Fe, and therefore cannot catalyse Fenton-type reactions yielding AOS. Up to now, the reason of AOS generation under Cd^{2+} stress is not consistent. First, Cd^{2+} can produce disturbances in the electron transport rates of photosystem Ⅰ and Ⅱ, leading to the production of AOS(Hegedüs *et al.*, 2001; Sandalio *et al.*, 2001); secondly, Cd^{2+} is known to trigger the oxidation of NADPH causing $O_2^{\cdot-}$ generation(Aravind *et al.*, 2003); thirdly, Cd^{2+} can disturb the function of antioxidant system resulting in $O_2^{\cdot-}$ and H_2O_2 accumulation(Luo, 1998; Schützendübel *et al.*, 2002) which is impossible in our study because SOD activity increases rapidly with the increase in $O_2^{\cdot-}$ generation. The generation of $O_2^{\cdot-}$ is probably due to the oxidation of NADPH which needs further confirmations.

SOD is located in various cell compartments and catalyze dismutation of $O_2^{\bar{}}$ or $HO_2^{\cdot}$ to H_2O_2 and O_2. Under natural physiological conditions, an increase in $O_2^{\bar{}}$ level can induce an elevation in SOD activity, however, the physichemical properties of SOD can be changed by H_2O_2 and OH, such as loss of Cu and Zn of Cu, Zn-SOD, and the inhibition in SOD activity increases with the increase in H_2O_2 level (Aravind *et al*. .2003; Fang *et al*., 2002). The increase in SOD activity in response to early Cd^{2+} stress is possibly attributed to the de-novo synthesis of the enzymic protein (Shah *et al*., 2001) besides the direct induction of $O_2^{\bar{}}$. However, long-term higher level of $O_2^{\bar{}}$ could increase H_2O_2 content and cause a marked decline in SOD activity, suggesting SOD has a limited function in scavenging $O_2^{\bar{}}$. Similar result has been obtained in tobacco leaves under Cd^{2+} stress (Yan *et al*., 1997).

The increase in leaf CAT activity similar to the report by Vitòria *et al*. (2001) is probably due to Cd^{2+} induced an increase in H_2O_2 content in peroxisomes (Romero *et al*., 1999). Contrary to leaf CAT, CAT activity in roots declined which may be explained as following: CAT enzyme is sensitive to $O_2^{\bar{}}$ and can be inactivated by its increasing levels (Aravind *et al*., 2003); A decrease in the protein content led to a decline in activity of CAT (Shah *et al*., 2001; Sandalio *et al*., 2001); POD are widely accepted as "stress enzymes", APX can also eliminate H_2O_2 and has higher affinity with H_2O_2 than CAT. A marked increase in GR activity will activate AsA-GsH cycle in roots, suggesting that an increase in APX activity is more probable in roots under Cd^{2+} treatment.

In a variety of organisms, ectopic over expression of SOD can cause an excess of the SOD activity relative to the H_2O_2 quenching activity, under this conditions, the activity of SOD may not be enough to scavenge all $O_2^{\bar{}}$, but may be sufficient to generate more H_2O_2 than in control, highly reactive ·OH would then be formed by the reaction of the remaining $O_2^{\bar{}}$ with H_2O_2. Increase in the ratio of SOD to CAT and POD activity, rather than individual changes in the activity of each enzyme, would lead to oxidative stress (Shan *et al*., 2001). Table 1 shows that the ratios of SOD/CAT in roots and in most of the cases in leaves increased with increasing Cd^{2+} toxicity, whereas the increase in GR activity may cause increase in POD activity thus the ratio of SOD/POD possibly did not show a definite change. Our results show that Cd^{2+} can induce oxidative stress through elevating the ratio of SOD/CAT in radish plants.

GR, a crucial enzyme in AsA-GsH cycle, reduces oxidized glutathione (GSSG) to GSH and plays an essential role in the protection of chloroplast against oxidative damage by maintaining a high ratio of GSH/GSSG (Pastori *et al*., 1992). Cd^{2+} can inactivate GR via directly or indirectly induced AOS generation (Schützendübel *et al*., 2002), and can also elevate GR activity due to the de-novo synthesis of enzyme protein (Vitòria *et al*., 2001; Dixit *et al*., 2001). GSH can be used to form PCs in higher plants. Ascorbate peioxidase (APX) increases following exposure to Cd^{2+} (Hegedüs *et al*., 2001; Dixit *et al*., 2001). Taking into consideration data above, the increase in GR activity would suggest AsA-GsH cycle may be activated to scavenge AOS and the synthesis of GSH may be stimulated for subsequent synthesis of PCs to chelate Cd^{2+} directly.

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